Fighting the HIV/AIDS Pandemic:

Developing Vaccines Aimed at Blocking HIV-1 Transmission

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Please view this presentation with the notes associated with each slide.
HIV-1 Replication Cycle

- Binding
- Membrane fusion
- Uncoating
- Reverse transcription
- Transport to nucleus
- Integration
- Viral gene expression
- Budding and maturation
Like other retroviruses that integrate their genomes into the genomes of their hosts, HIV initiate chronic infection within days of primary exposure to it. This leaves only a short window to get rid of the virus altogether.
HIV mutates quickly causing the immune system to lag behind it in attempts to generate effective immune responses to it.
HIV employs several tactics to evade the immune system including shielding critical protein components with a dense population of surface glycans and employing decoys.
Challenges Facing an HIV Vaccine

- **Retrovirus (latency) challenge**
- **HIV hyper-variability challenge**
- **Neutralizing antibodies challenge**
- **Correlates of protection challenge**
- **Antigen challenge**
- **Developing country challenge**

The “lucky few”:
- **Long Term Non-Progressors (LTNP)**
  - Genetics (Δ32 CCR5)
  - Auto-antibodies (anti CCR5)
- **Highly Exposed Persistently Seronegative** (HEPS)
  - Mucosal CTL
  - slgA responses - against gp41

Even the most deadly of human pathogens do not result in 100% mortality. Those that individuals that endured the infection by eliciting effective immune responses to the pathogen are extremely valuable for the design of an effective vaccine because they provide much needed correlates of protection. But for HIV there are no known cases of “recovery” from the infection, which makes vaccine design particularly challenging. Nevertheless, certain individuals potentially are resistant to HIV1 infection (HEPS) or to progression to AIDS (LTNP).
Challenges Facing an HIV Vaccine

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There is an almost strict inverse correlation between the prevalence and incidence of HIV1 infection and the growth domestic product of a country. Therefore for vaccines to have an impact they have to be inexpensive.
“[...] the primary focus of future studies for preventive strategies needs to be at the beginning of transmission - that is, at the mucosal barrier and the initial interaction of virus with host cells and host defenses.”  

“Host immunity has been linked to protection from infection in rare, highly exposed, persistently seronegative (HEPS) individuals. [...] the best hope for an effective vaccine must be to reproduce these responses as far as possible, and to induce them mucosally, at the site of subsequent viral exposure.”  

“The potential for complete (‘sterilizing’) immunity from HIV-1 infection may depend on the presence of pre-existing neutralizing antibodies.”  

“[...] new plant-based technologies have enormous potential for a variety of applications, including the oral delivery of vaccine antigens”.
(Koprowski and Yusibov. 2001. Vaccine 19:2735-41)
HIV-1 can gain access through the mucosal barrier by several ways:

(A) mucosal breach (e.g. caused by an underlying sexually transmitted disease.

(B) In pluristratified epithelium, HIV-1 can disseminate by attaching to dendritic cells.

(C) In simple epithelium with tight junction, transcytosis through the cells predominates. Transcytosis through M-cells (light blue) is also possible.
The **Membrane Proximal Region of gp41 (MPR\textsubscript{649-684})** “P1”

- Plays a critical role in host cell-virus membrane fusion
- Mediates transcytosis of virions
- Binds GalCer
- Is the target of transcytosis-blocking Abs (HEPS sIgAs, 2F5)
- Contains conserved epitopes for neutralizing Abs (2F5, 4E10)
- Spontaneously oligomerizes

**gp41-MPR\textsubscript{649-684}**

**SQTQEQEKLLELDKWASLNWFIDTNWLYIK**

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CTB-MPR649-684 may provide multivalent protections against HIV transmission and infection.

Cholera Toxin B Subunit (CTB)
- non-toxic subunit of cholera toxin
- a strong mucosal adjuvant
- pentamer
- binds to GM1 ganglioside on M cells

Image by JD Clemens
CTB-MPR\textsubscript{649-684} may provide multivalent protections against HIV transmission and infection.

1. Block transcytosis across epithelial cells
2. Neutralize virus particles and prevent infection of CD4\(^+\) cells
3. Destroy HIV-infected CD4\(^+\) cells
4. Neutralize virus particles and prevent infection of CD4\(^+\) cells

CTB-MPR

CTU

S-IgA

IgG

G\textsubscript{M1} ganglioside

HIV-1-infected CD4\(^+\) cells

Antigen Presenting Cells

Th1

Th2

CD4\(^+\) cells

B

CTL

HIV-infected CD4\(^+\) cells

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Expression of CTB-MPR$_{649-684}$ in *E. coli*

1. Coated with:
2. Overlaid with:
3. Detected with:

- CTB
- CTB-MPR
- MPR
- 2F5 or 4E10
- Anti-CTB

Non-denaturing conditions

CTB-MPR fusion
- retains $G_{M1}$-binding
- presents 2F5 and 4E10 epitopes.

Expression of CTB-MPR$_{649-684}$ in *E. coli*

- pelB signal
- CTB-MPR$_{649-684}$ gene
- His tag
- T7 terminator

Nco I  Nde I  Xho I

- T7 promoter
- T7 terminator

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- retains $G_{M1}$-binding
- presents 2F5 and 4E10 epitopes.
CTB-MPR binds to both GM1 and GalCer

Ligand Blot Analysis

GM1  GalCer  PC

CTB: GM1 binding

MPR: GalCer binding

M cell  Epithelial cell

Antigen Presenting Cell  Target cell

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Quantitative Analysis of GM1-Binding Affinity

CTB-MPR retain the same binding affinity to GM1 as native CTB

<table>
<thead>
<tr>
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<th>IC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
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<tr>
<td>CTB-MPR</td>
<td>60.7</td>
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<tr>
<td>CTB</td>
<td>57.3</td>
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Average of 2 experiments
Immunization of mice with CTB-MPR

CTB-MPR successfully induced anti-MPR Abs

Serum IgG

Fecal IgA

Vaginal IgA

First, effect of the anti-P1 antibodies on HIV transcytosis was evaluated in vitro HIV transcytosis assay using human tight epithelial model. In this system, human epithelial cell lines are cultured to form monolayer with tight junction in the upper chamber. Once HIV-infected CD4+ cells are added to the top chamber, HIV particles start budding from the infected cell and intact viruses are transcytosed across the cell barrier which you can detected in the bottom chamber.
HIV transcytosis assay using human tight epithelial model


Anti-MPR Abs blocked HIV-1 transcytosis
Next, we tested if anti-P1 Abs can prevent HIV infection to human cells in HIV neutralization assay. In this system, primary R5 type HIV-1 isolate and human peripheral blood mononuclear cells were used. Once the PBMCs were cultured with the virus, the cells are infected which can be detected within a few days.
Now, in the presence of neutralizing Abs, the cells are prevented from infection. Here are the results when sera of mouse immunized with CTB-P1 were added to the culture. Unlike non-immune control serum, these sera were shown to inhibit PBMC infection, as high as 90% inhibition was obtained at 1:10 dilution. These results indicate that anti-P1 Abs can not only block the HIV transcytosis but also prevent the infection. Remember that P1 contains epitopes of broadly neutralizing mAbs 2F5 and 4E10. Although this is still preliminary data and we need to verify this effect using a broad spectrum of HIV isolates, to our knowledge CTB-P1 is the first successful example of antigen based on neutralizing mAb epitopes that can induce neutralizing Abs.
CTB-MPR_649-684 as a mucosal subunit vaccine against HIV-1 infection/transmission

Matoba et al, PNAS 2006
CTB-MPR expression in tobacco (*N. benthamiana*)

Agrobacterium

E. coli

CTB-MPR

CTB

Tobacco

Anti-CTB

2F5

KDa

130 73 54 35 24 16 130 73 54 35 24 16
Immunogenicity of Plant-derived CTB-MPR in Mice Primed with the Antigen Produced in *E. coli*

Plant-derived CTB-P1 is immunogenic and immunologically cross-reactive with the antigen produced in bacteria
Towards inexpensive oral vaccination

1. CTB-MPR649-684 expression vector
2. Agrobacterium
3. Cotyledons
4. Shoots
5. A rooting transformant
6. USDA regulated plant production
7. Freeze-dried fruits
8. FDA regulated GMP
9. Product
Crystalization and X-ray diffraction studies may help design a more effective immunogen.
Summary

1. A fusion protein comprising the gp41 membrane proximal region fused to CTB was designed.

2. The chimeric protein can be produced in bacteria and purified to electrophoretic homogeneity.

3. The chimeric protein can be crystallized.

4. Best immunization strategy will involve a prime-boost approach with mixed-route and two different carrier proteins.

5. Transgenic plants can produce CTB-MPR_{649-684} structurally and antigenically comparable to the *E. coli* produced protein.
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